

# The relationship between IL-16 and Foxp3 expression in Treg cells of healthy individuals

The relationship between serum IL-16 levels and Foxp3

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## Abstract

**Aim:** It has been demonstrated that Interleukin-16 (IL-16) is a protein that has specific effects on CD4+ T lymphocytes and is secreted from many immune and non-immune cells. Regulatory T cells (Tregs) are cells that prevent the development of autoimmune diseases and transplant rejections. It is thought that Tregs may also play an important role in the development of allergy and asthma. Tregs can control the immune response by inhibiting the function of other cells involved in the immune response. FoxP3 is a transcription factor that regulates the activation of Tregs. In this study, we aim to determine whether there is a correlation between serum IL-16 levels and FoxP3 expression in CD4+CD25+ mononuclear cells in healthy individuals.

**Material and Methods:** While IL-16 levels were analyzed with the ELISA method, FoxP3 levels were measured by flow cytometry in peripheral mononuclear cells, in serum samples obtained from 30 healthy volunteers. The Spearman correlation test and the SPSS 15.0 statistical program were used to evaluate the relationship between serum IL-16 and FoxP3 Expression MFI.

**Results:** The average serum IL-16 levels were 151.9 pg/ml (89.4-230.6). A positive correlation was observed between serum IL-16 and FoxP3 expression. To the best of our knowledge, the in-vivo relationship between IL-16 and the Treg cells was demonstrated in this study.

**Discussion:** High serum levels of IL-16 are observed in the disease groups with atopy, allergy, and autoimmunity, where the immune system is deficient or malfunctioning, which may contribute to the comprehensibility of the IL-16-disease relationship and may clarify the relationship between Treg-IL-16.

## Keywords

Treg, FoxP3, IL-16, CD4

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## Introduction

Interleukin-16 (IL-16) was first described in 1982 as the result of increasing chemotactic activity of T lymphocytes by stimulating peripheral blood mononuclear cells with mitogens. During the inflammatory response, IL-16 is released from mast cells, eosinophils, dendritic cells, T lymphocytes, and certain stromal cells. While IL-16 was previously known as a lymphocyte chemotactic factor, today, it has been proven to be a cytokine with specific effects on CD4<sup>+</sup> T lymphocytes, specifically, it has been shown to be a cytokine released from CD8<sup>+</sup> T lymphocytes as a result of stimulation of histamine and serotonin. IL-16 has two primary functions in the immune system. Its first function is that it is a chemoattractant factor of CD4<sup>+</sup> cells. The other important task of IL-16 is to suppress the release of Th2 type cytokines (IL-4, IL-5, IL-13) as a result of antigenic stimulation and to increase the production of interferon-gamma (IFN-gamma) [1, 2]. In addition, IL-16 plays a role in the expression of IL-2R and MHC class-II of resting CD4<sup>+</sup> T cells and selectively inhibits chemokine stimulation. In asthma patients, IL-16 has been shown to play a role in reducing or preventing the inflammatory response. It was observed that, while IL-16 knockout mice showed to be hyperresponsive to experimental lung changes and increased inflammation, parenteral or aerosol administration of IL-16 to normal mice inhibited lung hyperresponsiveness, goblet cell hyperplasia, and inflammation [3]. IL-16 is associated with the inflammatory response observed in asthma, rheumatoid arthritis, systemic lupus, erythematous, colitis, atopic dermatitis, and multiple sclerosis [4, 5]. The fact that IL-16 affects the functions of CD4<sup>+</sup> T cells and causes IL-2R expression in these cells suggests that it is a pro-inflammatory immune-regulatory cytokine. Regulatory T cells (Tregs) are cells that prevent the development of autoimmune diseases and transplant rejections. Tregs are able to control the immune response by inhibiting the function of other cells involved in the immune response. Specific markers of CD4<sup>+</sup> CD25<sup>+</sup> Treg cells were detected. These are the glucocorticoid-induced tumor necrosis factor (TNF) receptor and the FoxP3 [6]. This study aims to examine the relationship between the expression of IL-16, which plays an important role in the immune system and acts through CD4<sup>+</sup>, and FoxP3, which plays a role in Treg cell functions. For this purpose, the serum IL-16 levels and the FoxP3 expression levels in CD4<sup>+</sup>CD25<sup>+</sup> mononuclear cells of volunteer participants were evaluated.

## Material and Methods

### Study Groups and Blood Samples:

The study included a total of 30 healthy individuals, 17 males and 13 females aged between 19 and 35 years. Participants were carefully evaluated for any history of allergy and autoimmunity and whether they had any infections in the last 10 days. Complete blood counts of all participants were normal. Before participating in the project, all participants were informed in detail, and they all signed an informed consent form (a master thesis project by the Health Sciences Institute Board Date and No: 2006.02.0122). The blood sample was taken in a sterile environment by entering a vein of suitable diameter on the forearm, in a vacutainer 2 ml straight tube, in a vacutainer 10 ml heparin tube, and in a vacutainer 3 ml

EDTA tube. A complete blood count was performed on blood samples taken into tubes containing EDTA, mononuclear cells were prepared from the heparinized blood samples. Serum was separated from the blood that was drawn into straight tubes and subsequently stored at -800 C to check the IL-16 level.

### Peripheral Blood Mononuclear Cell Preparation

Blood samples from 30 healthy adult volunteers, 10 ml each, collected in vacutainer heparin tubes, were assayed in a sterile environment. Mononuclear cells were added to sterile 15 ml tubes on 3 ml of ficoll-isopaque (specific gravity 1.077 g/ml) by slowly adding 5 ml of peripheral blood (careful not to mix the Ficoll with the blood). This was obtained after centrifugation for 30 min at 400 G. Obtained cells were washed 2 times with sterile PBS and prepared for immunophenotypic analysis.

### Determination of the Serum IL-16 Levels

After the serums that were stored in -800 C were thawed at room temperature, the following steps were followed using the IL-16 ELISA Kit (BioSource Human IL-16 ELISA). In summary, 100 µL of standard serum controls were added to the wells. Antigen-specific Biotin Conjugate (50 µL) was added onto it. The wells were incubated at 25°C for 3 hours. At the end of incubation, the wells were emptied and washed 4 times with wash buffer. Streptavidin-HRP conjugate (100 µL) was added to each well. It was incubated at 25°C for 30 minutes. The liquid in the wells was drained and washed 4 times with wash buffer. Stabilized chromogen (100 µL) was added to each well. The liquid in the wells was drained and washed 4 times with wash buffer. It was incubated at 25°C for 30 minutes, and 100 µL of stop solution was added. The absorbance was read at 450 nm.

### Measuring of the FoxP3 Expression Level

FoxP3 expression was measured with the flow cytometric method. Before the analysis, mononuclear cells were immunophenotyped using the following antibodies. Antibodies used for isotype control: anti-IgG1-FITC, anti-IgG2-PE, anti-IgG1-APC; antibodies used to detect Treg cells: anti-CD4-FITC, anti-CD25-PE, anti-FoxP3-APC. Flow cytometry analysis was performed after the immunophenotyping. Analyzes were performed at Akdeniz University Central Laboratory, using the Becton Dickinson (FACS Calibur) flow cytometry device. Fragmented cell debris and electronic contamination were removed by adjustment via forward scatter. First of all, the distribution of cells according to their size and granularity is shown, mononuclear cells were covered with the forward and 90 0 side scatter histogram, and analyzes were performed on this region. To detect the expression of the monoclonal antibody of interest, regions showing more intense fluorescence than the fluorescence of the negative isotopic control were determined from the particles in the analysis region. The percentage of the entire population of the target cells and the mean fluorescence intensity were evaluated separately when performing the analysis. Analyzes were performed with the CellQuest software.

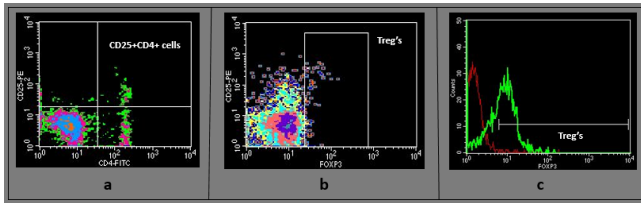
## Results

### 1. CD4<sup>+</sup> CD25<sup>+</sup> FoxP3<sup>+</sup> Cell Ratios

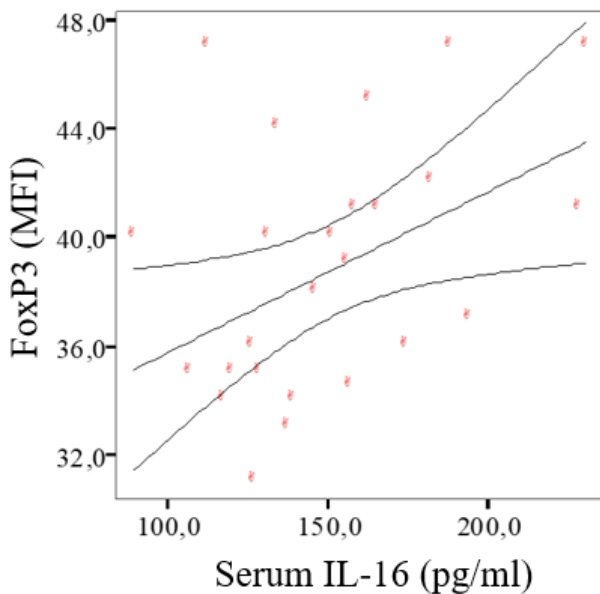
After the mononuclear cells were isolated from the blood samples of 30 healthy volunteers, CD4<sup>+</sup>CD25<sup>+</sup> FoxP3<sup>+</sup> cells were evaluated flow cytometrically (Figure 1). The mean was found to be 1.1% (0.9-1.3).

## 2. Comparison of Serum IL-16 Levels and FoxP3 Mean Fluorescence Intensity (MFI) of CD4+ CD25+ FoxP3+ Cell Population

Serum IL-16 levels were found to be 151.9 pg/ml (89.4-230.6) on average, when measured for the first time, a statistically significant correlation was found between the FoxP3 mean fluorescence intensity (MFI) of the CD4+ CD25+ FoxP3+ cell population ( $p < 0.05$ , Figure 2). This result shows us that the CD4+ CD25+ FoxP3+ cell population and the mean fluorescence intensity of FoxP3 expression are high in individuals with high serum IL-16 levels.



**Figure 1.** Flow cytometric representation of CD4+CD25+FoxP3+ cells. a) CD4+CD25+ mononuclear cells, b) CD4+CD25+FoxP3+ dot plot of Treg cells, c) CD4+CD25+FoxP3+ histogram display, the red line represents isotypic control, the green line is a sample.



**Figure 2.** Comparison of FoxP3 MFI and serum IL-16 levels of the first-time CD4+CD25+FoxP3+ cells ( $p < 0.05$ ).

## Discussion

Interleukin-16 (IL-16) is a cytokine that is a natural ligand of CD4. Sites of allergic inflammation have been identified in both the animal and human airway epithelium and bronchoalveolar lavage specimens. It is a known chemoattractant factor for CD4+ lymphocytes, monocytes, eosinophils, and dendritic cells, and it exhibits selective chemoattractant activity for the Th1 subgroup of CD4+ cells. It also increases the expression of CD25 (IL-2R) on CD4+ cells. In vivo studies suggest that administration of IL-16 results in a reduction in inflammation and airway hyperreactivity. The mechanism of this regulatory effect on inflammation has not yet been fully explained [7]. There are also studies showing that IL-16 plays a vital role

through its effect on the pathogenesis of CD4+ T cells in the immune/inflammatory diseases such as allergic rhinitis [9]. Some researchers have reported that IL-16 may also accompany the pathogenesis of diseases such as asthma [4], rheumatoid arthritis, systemic lupus erythematosus, colitis, atopic dermatitis, and multiple sclerosis [8,9]. Regulatory T cells (Tregs) control the immune response by inhibiting the function of other cells involved in the immune response. They are divided into two main groups. The first group was named natural CD4+ CD25+ regulatory T (n Treg). The other group is the cell type called adaptive Treg, these cells form under certain conditions in the periphery [12]. FoxP3 is the specific marker of Tregs. Studies in both human and FoxP3-deficient mice indicate that this transcription factor plays an important role in the regulation of T cell function [13].

In this study, the chemotaxis effect of IL-16 on the CD4+ T cells, which is also an activation marker of CD4+ CD25+ T cells on the FoxP3 expression, was examined. In our study, conducted with 30 healthy individuals, the mean serum IL-16 level was found to be 151.9 pg/ml (SD: 33.4 Min: 89.4 Max: 230), and there was a statistically significant relationship ( $p < 0.05$ ) between FoxP3 MFI in the first CD4+ CD25+ FoxP3+ cells. FoxP3 MFI was found to be high in individuals with high serum IL-16 levels. To our knowledge, this is the first in vivo report showing that IL-16 has a positive effect on FoxP3, which is the activation marker of Tregs. As a result, there is a positive correlation between serum IL-16 and FoxP3 expression. We believe that our study may contribute to the pathophysiology of diseases such as atopy, allergy, and autoimmunity, in which the immune system is deficient or malfunctioning, and to understanding the unclarified mechanism of IL-16 in the immune response.

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## Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

## Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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## Conflict of interest

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